STUDIES ON THE ELASMOBRANCH KIDNEY. IV. THE SECRETION OF PHENOL RED BY THE SMOOTH DOGFISH, MUSTELUS CANIS [1]

RUDOLF T. KEMPTON

*Department of Biology, Vassar College, Poughkeepsie, N. Y.,
and the Marine Biological Laboratory, Woods Hole, Mass.*

While the reabsorptive process of the kidney of the smooth dogfish has been studied in relation to urea (Kempton, 1953), tubular secretion has not been examined. The present study was initiated to determine to what extent these renal tubules are capable of secretory activity.

A further consideration is the fact that in recent years increasing use of *Mustelus canis* as an experimental animal has resulted in one body of information concerning the smooth dogfish and another for the spiny dogfish (*Squalus acanthias*), with little work being done on both species. Although the similarity in popular names suggests a close relationship between the two animals, they are in fact placed in different primary subdivisions of the sharks (Bigelow and Schroeder, 1948). Since phenol red elimination has been studied in the spiny dogfish by Willie Smith (1939) a study of this process in the smooth dogfish permits comparison of the two species.

MATERIALS AND METHODS

Female smooth dogfish weighing 3.5 kg. or more were kept untreated in running sea water for approximately 24 hours after their capture by otter trawl. This gave time for recovery from the effects of capture and transportation. On the day preceding their use they were weighed and injected intravenously with inulin (10 ml./kg. of a 10% solution freshly prepared with distilled water). The following morning an in-lying catheter was tied into the urinary papilla, a balloon was attached to collect urine, a blood sample was taken and the animal was injected with phenol red. The usual dosage was 5.0 ml./kg. of an 800 mgm.% solution in 1% $NaHCO_3$ administered intravenously through the caudal vessel. A period of two to three hours elapsed before starting the collection of urine, in order to insure sufficient time for distribution of the dye. In a few cases the dosage was greater, the largest being an intravenous injection of 5 ml./kg. of a 2400 mgm.% solution of phenol red, accompanied by 2.0 ml./kg. injected intramuscularly. Even the standard dosage gave a deep red color which disappeared gradually over 12–36 hours. Repeated collection periods were made until the blood level of phenol red was too low for accurate determination or some untoward event caused the termi-

nation of the experiment. Usually the periods were 3–4 hours in duration, except for one 6–8-hour period overnight. For each period the plasma level of phenol red and inulin was taken as the average of the concentrations at the start and end of the period. Urine was collected with the aid of abdominal massage.

The method of collecting urine and the analytical method for inulin were as described previously (Kempton, 1953). Phenol red determinations were made in a Coleman Junior Spectrophotometer at 560 millimicra, after the blood and urine were diluted to range with 5% $NaHCO_3$. At lower phenol red concentrations even a very small amount of scatter would cause significant error. Such scatter was compensated by use of a blank consisting of plasma which had been collected previous to dye injection, and which had been diluted with $NaHCO_3$ solution to the same extent and at the same time as the plasma containing phenol red. With higher plasma concentrations, and with urine, the blank was merely 5% $NaHCO_3$. Suitable phenol red standards were prepared in 5% $NaHCO_3$.

During the summers of 1962 and 1963 all the animals were kept at the temperature of the sea water system at the laboratory, namely 20° C. ± 2°; in the latter part of the summer of 1964 some of the animals were kept in running sea water which had been chilled to between 12° C. and 16° C. In this colder water the fish had a markedly lowered rate of urine flow.

A total of 100 successful collection periods was obtained over the three summers. Clearances were calculated from the volume of urine flow and the concentration of phenol red and inulin in the plasma and urine. Other calculations were made to determine possible causal relationships.

In making some of these computations it was desirable to take into consideration the binding of phenol red to plasma proteins. Pooled samples of heparinized smooth dogfish blood were separated by centrifugation. A phenol red stock solution was prepared by dissolving dry phenol red in some of this plasma. This was added to other aliquots of plasma in varying amounts to give concentrations of phenol red in approximately the same range as that found in the experimental animals. Some of the dye-free plasma was retained for use in blanks during the determination of phenol red concentrations. Each dye-containing plasma sample was filtered under oil through a dialyzing membrane[2] at a negative pressure of 120–130 mm. Hg, the filtrate being collected under oil. The dye concentration of the plasma was compared with that of its filtrate. The average of 15 determinations indicated that 89.3% of the plasma phenol red is filterable in the range of experimental plasma levels. This value was used in the calculations of the rate of phenol red filtration. Since this factor as applied produces only a 9% reduction in the calculated filtration rate, any small error in the value would have little significance in the computations.

RESULTS AND DISCUSSION

When the total phenol red excretion per ml. of filtrate was plotted against plasma concentration (Fig. 1), the excretion rate increased rapidly as the plasma levels increased. In the graph tubular secretion is represented by the distance between a circle on the upper curve and the corresponding square on the lower; the amount

---

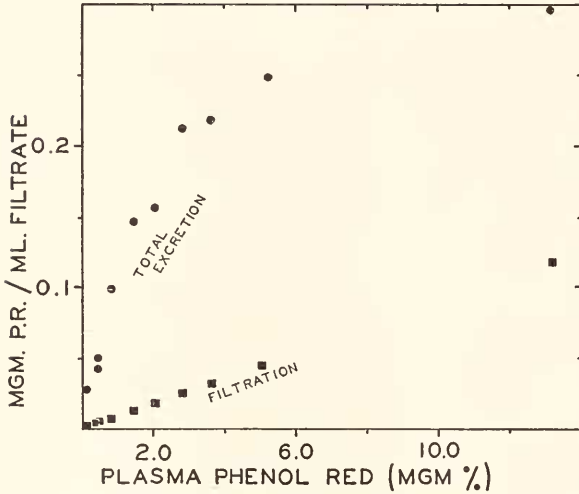[2] Arthur H. Thomas Co., Philadelphia, catalogue no. 1165–A2.

FIGURE 1. Relation between varying concentration of phenol red in the plasma and the amount of dye excreted per milliliter of filtrate. In this and other figures each point represents an average of 10 collection periods, based on increasing plasma phenol red concentrations. Circles = total excretion; squares = calculated filtration.

of dye filtered is represented by the distance from the square to the baseline. Tubular secretion thus became roughly constant at plasma levels above 2–3 mgm.%. The ratio between the clearances of inulin and phenol red is shown in Figure 2. Since the clearance of inulin represents the rate of filtration, ratios above unity depend on tubular secretion. Thus, there was a significant amount of secretion at all plasma levels, but it was especially great at the lower concentrations. The fraction of the excreted dye which was secreted by the tubules (Fig. 3) ranged from an average of 94.8% in the 10 periods in which the plasma phenol red was
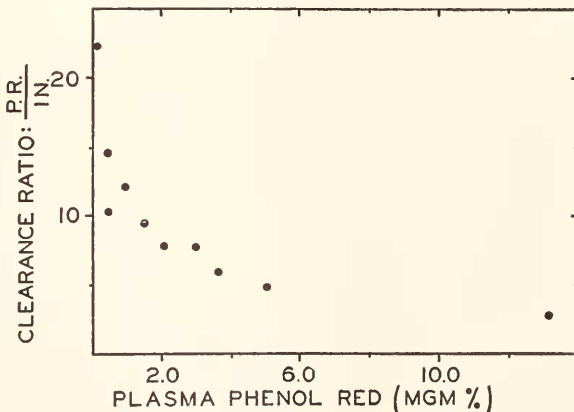

FIGURE 2. Relation between varying concentration of phenol red in plasma and the ratio of the clearances of inulin and phenol red.
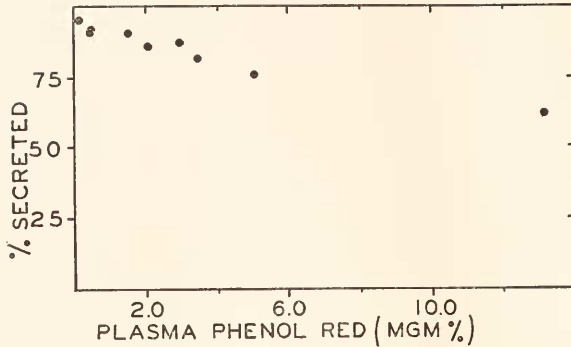
FIGURE 3. Percentage of the excreted phenol red which was eliminated by tubular secretion at varying plasma levels.

lowest, to an average of 61.7% in the 10 periods in which the plasma load was greatest. As the plasma level of phenol red was increased, the fall in the secreted fraction was essentially linear.

Relating excretion to units of filtrate as above eliminates the effects of variation in the volume of filtrate. The absolute rate of secretion (mgm./hr./kg.) was much more variable. At low plasma concentrations the amount of secretion rose rapidly with increased levels of phenol red (Fig. 4), reaching a maximum secretory rate when the plasma phenol red was between 2 and 3 mgm.%. However, this maximum rate was not maintained as the plasma level increased, but fell to a little less than 50% of the maximum. This will be discussed below.

The amount of secretion of phenol red was sufficiently great to lead to a very high urine/plasma (U/P) ratio when the plasma levels were low, and this ratio fell rapidly as the plasma levels increased (Fig. 5). Since the decrease in tubular
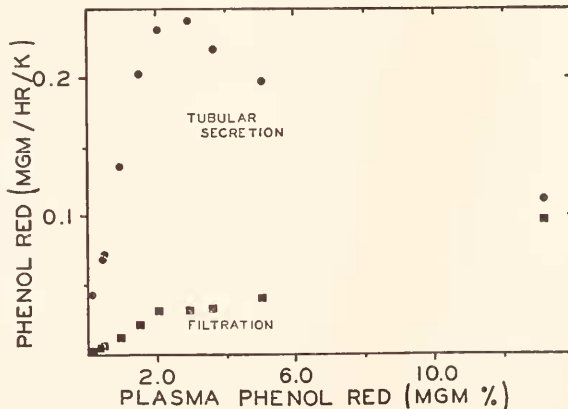

FIGURE 4. Total amount of tubular secretion of dye and of filtration at varying plasma concentrations. Above a plasma level of 2.0 mgm% the filtration rate was less than would have been expected on basis of unchanged filtration volume. Circles = tubular secretion; squares = filtration rate.

secretion appeared only at plasma levels above 2–3 mgm.%, it is clear that the first part of the fall in U/P ratio was not dependent on decreased secretion.

The U/P ratio of inulin indicates the degree of concentration due to the reabsorption of water from the filtrate. Although there was a very gradual fall of nearly 50% in the inulin U/P ratio, starting at a phenol red plasma level of 2–3 mgm.%, the U/P ratio of phenol red was obviously of a different order of magnitude; and unlike the inulin U/P value it was very responsive to changes in plasma phenol red level.

The interpretation of the data is obscured by the fact that in these experiments the rate of filtrate formation (inulin clearance) was depressed at the higher phenol red levels. The cause of this is not clear, but it could be due to the high phenol red levels, to the large amount of $NaHCO_3$ in the vehicle for phenol red, or to the lower
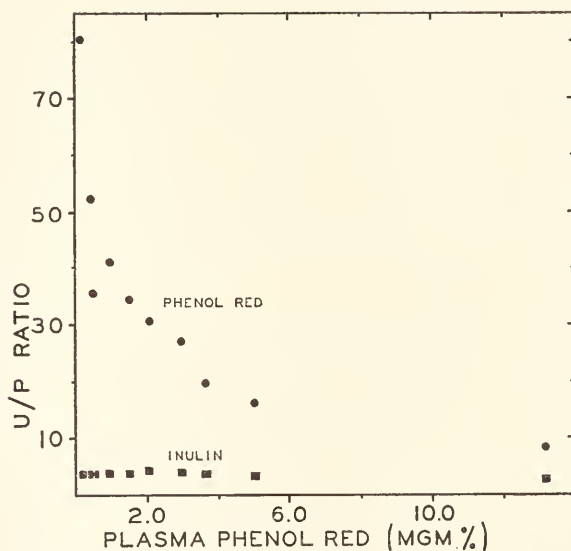

FIGURE 5.   U/P ratios of phenol red and inulin at varying plasma phenol red levels.
Circles = phenol red U/P; squares = inulin U/P.

water temperatures which prevailed during several of the periods. Regardless of cause, this depression is seen in Figure 6, in which the filtration rate (inulin clearance) values are somewhat scattered but clearly became lower when the plasma levels of phenol red were above approximately 2 mgm.% The depression of total water reabsorption, represented in the same figure, is considered to be largely a consequence of the reduced filtration. It is clear from the data illustrated in Figure 7 that a relatively constant proportion of the filtered water was reabsorbed. This fraction varied only from a minimum of 60% to a maximum of 75%, the variations following no marked trend. In contrast the amount (ml./hr./kg.) of water reabsorbed and the amount of urine produced (Fig. 6) were much more variable than this.

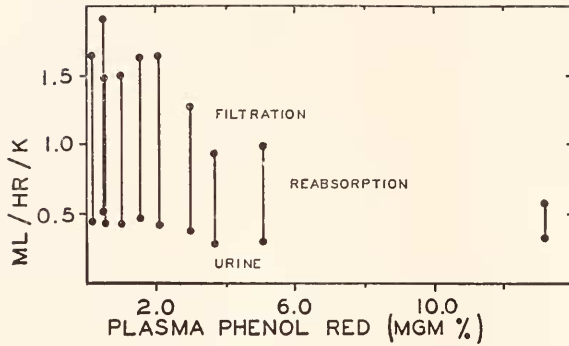The observation that at plasma levels higher than approximately 3 mgm.%

FIGURE 6.   Volume of filtration, reabsorption and urine flow
at varying plasma levels of phenol red.

phenol red there was a fall (Fig. 4) in total secreted phenol red (mgm./hr./kg.)
raises a question as to its nature.   It is clear that there was also a fall in the rate of
formation of filtrate.   Not only did this appear in the inulin clearance ("filtration")
of Figure 6 but logically it would have to be true even though that evidence were
not available.   This follows from the fact that with increasing plasma levels the
phenol red concentration in the filtrate automatically becomes greater and it is only
through reduction in volume that the total amount of filtered dye could be decreased
as plasma levels rise.

A reduction in the rate of filtrate can result from two different causes, acting
separately or together.   First, decreased total filtration could result from a reduction
in the filtration by the individual glomeruli (perhaps as a result of reduced intra-
capillary pressure).   Alternately, the same result could be obtained by a complete
cessation of function of some glomeruli while others continued with unimpaired
activity.   The data appear to give a basis for choosing between these two explana-
tions of the present results, because the two possibilities have different sequelae.

If the reduced total filtration were due to a reduction in filtration of all indi-
vidual glomeruli, there would be several consequences.   (1) There would be a
reduction in the total amount of filtered dye.   Figure 4 shows that this was the
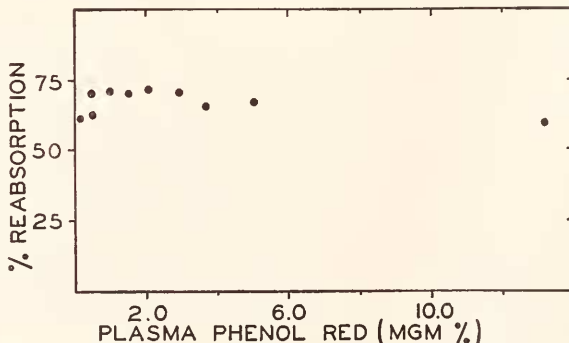

FIGURE 7.   Percentage of water reabsorbed from the filtrate at varying plasma phenol red levels.

case, in that the increase in dye filtration was inadequate to keep pace with the expectations based on the increasing concentration of dye in the plasma and filtrate. (2) In the reduction in the total volume of urine-plus-reabsorption, changes of reabsorption obviously cause changes in urine rate. Thus, in Figure 6 while the filtration rate continued to fall with increased phenol red plasma levels, a reduction in the water reabsorption kept the urine rate from falling to even lower levels. (3) The effect that decreased glomerular filtration would have on total phenol red tubular secretion depends on whether the cells were secreting maximally. Since the fall in filtration which is being considered took place at or above levels at which tubular secretory maximum had been reached (Fig. 8), discussion can be confined to this special case. (4) With the cells secreting maximally, a slower rate of progress along the tubule should lead to higher rather than lower U/P ratios, since more time would be available for the higher concentration to be reached. In the event that the concentration ratio should become the limiting factor in secretion, there would be an upper limit of concentration ratio, but the ratio should not fall.
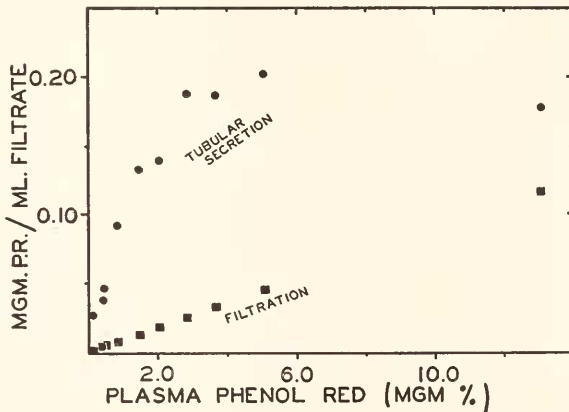

FIGURE 8.   Tubular secretion related to unit volume of filtrate at varying plasma phenol red concentrations.   Circles = tubular secretion; squares = filtration.

In fact the ratio did continue to fall in a consistent fashion (Fig. 5) which gives no indication of an upward tendency.

With the other possibility (complete cessation of function of some glomeruli, and continued unchanged function of the others) the effect on total filtration volume and total phenol red filtration would be no different from the first case. But there is a clear difference between the two situations. If the functioning glomeruli were unimpaired, decreased filtration and consequent decrease in the amount of water reabsorbed should have no effect on the percentage which is reabsorbed. This is because each glomerulus and tubule which is still functioning is behaving as it did previously, producing and processing the original amount of filtrate. As a matter of fact, the percentage of water reabsorption remained surprisingly constant (Fig. 7). When phenol red is considered, it is clear that the filtrate which formed under these conditions would contain an unchanged concentration, but a

reduced total amount of phenol red would be filtered in view of the decreased number of filtering glomeruli. In those tubules whose glomeruli were still functioning, the processes of filtration, reabsorption and secretion would be unchanged. The non-functioning glomeruli and their tubules would simply be subtracted from the picture. Therefore there should be no change of U/P ratio related to the change in rate of filtration. The smoothness of the curve in Figure 5 shows this to be the case.

Another consideration also points toward the elimination of functional glomeruli as the cause of both the reduced total secretion of phenol red, and the reduced glomerular filtration. If secretion is considered in terms of mgm./hr./kg. there is a marked fall in secretory rate with plasma levels above 3 mgm.% (Fig. 4). On the other hand, if secretion is expressed in terms of each milliliter of filtrate produced, this fall either does not appear or is very much reduced (Fig. 8). The explanation appears to be that the absolute amount of secretion is the total of all the glomeruli and tubules, whether active or inactive. But when secretion is considered in relation to filtrate volume, only the active glomeruli and tubules are being considered, and these still active tubules continue to behave in an unchanged manner. Thus, the difference between Figures 4 and 8 offers evidence that the reduction in filtration is due to a reduced number of glomeruli rather than to reduced function of individual glomeruli.

One further consideration points in the same direction without involving phenol red. Figure 6 indicates that above the critical phenol red plasma concentration of 2–3 mgm.% the rate of filtration falls about 50%, urine production falls about 50%, total reabsorption of water falls about 50%; while on the other hand the *percentage* of water which is reabsorbed falls very little (Fig. 7). Such an agreement is compatible with a situation in which some of the glomeruli and tubules are removed from the over-all function while the remainder continue to behave as before. No other explanation seems to fit the facts with equal cogency.

A comparison of the data derived from these experiments with some of those found by Smith in the spiny dogfish, is summarized in Table I:

TABLE I

Comparison of the smooth dogfish (Mustelus canis) and the spiny (Squalus acanthias)
(Smith 1939)

	Smooth	Spiny
Phenol red clearance (cc./kg./day)	593	1750
% phenol red secreted	91.7%	99%
Clearance phenol red / Clearance inulin	14.8	22.5
Tubular maximum mgm./kg./day	5.8	18.1
Inulin clearance (cc./kg./day)	40	78

It seems clear from these data that the general behavior of the kidneys of the two species is similar. At plasma dye levels below 1 mgm.% the phenol red clearance, the percentage of the excreted dye which is actively secreted by the

tubules, and the ratio of the clearances of inulin and phenol red are all somewhat lower in the smooth dogfish than in the spiny. The tubular maximum, which seems to be reached at about the same plasma level in the two species (2–3 mgm.%), is lower in the smooth dogfish. The fact that in all these features the values in the smooth dogfish are lower than in the spiny suggests the possibility that the relation between the mass of effective kidney tissue and total weight may be somewhat different in the two animals, the larger (smooth dogfish) on this basis having less excretory tissue in relation to the whole.

Summary

1. With increasing plasma concentrations of phenol red, starting at very low levels, there is a rapid and extensive increase in its excretion, reaching an asymptote at approximately 10–11 mgm.%. This is clearest when the data are so treated as to neutralize the effect of variations in the rate of filtration.

2. Much more phenol red is secreted by the tubules than is filtered by the glomeruli. The percentage of excreted dye which was eliminated by tubular secretion varied from a maximum of 96% at the lowest measurable plasma levels to a minimum of about 60% at the highest blood levels obtained. The decrease was essentially linear.

3. At low plasma levels the ratio of the clearance of phenol red and inulin was approximately 25; at the highest plasma levels the phenol red clearance was reduced to only about three times that of inulin.

4. The U/P ratios were of a very different order of magnitude. That of inulin remained relatively constant at a level between 2 and 5; while with increasing plasma levels the phenol red U/P ratio fell from an initial 60 to a minimum of approximately 6. A fall below 1.0 has been reported for the spiny dogfish but this was at plasma phenol red levels far above those achieved in the present experiments.

5. In this series of experiments the rate of phenol red filtration and the rate of phenol red secretion both became reduced at the higher blood levels of phenol red.

6. With decreasing rates of filtrate formation, a reduction in the rate of water reabsorption prevented rates of urine formation from falling to even lower levels than they did.

7. While at higher plasma levels of phenol red there was a decrease in total water reabsorption, there was very little change in the percentage of the filtrate which was reabsorbed.

8. Data produced by these experiments indicate that the reduced filtrate formation at higher plasma levels was due primarily to a complete cessation of the function of some glomeruli rather than to a mere reduction of filtration rates of all the glomeruli.

9. Comparison of these data on *Mustelus* with those existing in the literature on *Squalus acanthias* indicates that the two kidneys behave in much the same fashion. The fact that most measurable factors were relatively lower in *Mustelus* suggests that the total excretory mass may be smaller in relation to the total weight in this animal. It is possible also that some of this difference might disappear if the two species were compared on the basis of body surface rather than body weight.

LITERATURE CITED

BIGELOW, HENRY B., AND WILLIAM C. SCHROEDER, 1948. Fishes of Western North Atlantic. Part one: Lancelets, Cyclostomes, Sharks. Memoir #1 of the Sears Foundation for Marine Research.

SMITH, WILLIE W., 1939. The excretion of phenol red in the dogfish, *Squalus acanthias*. *J. Cell. Comp. Physiol.,* **14**: 357–363.

KEMPTON, RUDOLF T., 1953. Studies on the elasmobranch kidney. II. Reabsorption of urea by the smooth dogfish, *Mustelus canis*. *Biol. Bull.,* **104**: 45–56.